Eight Steps to Method Validation in a Clinical Diagnostic Laboratory

Abstract

Method validation is utilized to confirm that a test procedure for an analyte yields accurate and precise results. In this article, hemoglobin A1C is used as an example to demonstrate the process of a method validation; the performance of the Siemens Dimension Vista 1500 (new method) was compared to the Integra 800 Roche Modular System (old method). There are eight essential components for method validation: stating the primary objectives, listing the known variables, applying statistics, clarifying the analyte involved, selecting samples, explaining the methods used, performing data analysis, and explaining the results. These steps are critical for an analytical method validation procedure and are required in order to use a new assay for the clinical diagnosis of a patient.

Abbreviations:

CAP – College of American Pathologists, CLIA – Clinical Laboratory Improvement Amendments, HbA1C – Hemoglobin A1C, R – Correlation Coefficient, SE – Systematic Error, SD - Standard Deviation, SEa - Allowable systematic error, RE – Random Error, TEa – Total Allowable Error

Index Terms:

Method validation, Precision, Accuracy, Linearity, and Correlation.

Learning Objectives:

1. Define method validation.

2. Elucidate the eight steps involved in a method validation.

3. Explain how statistics is applied to validate a new method.

Introduction

Method validation is the process used to confirm the accuracy and precision of a given analytical method or instrument. The true value of a patient-derived analyte depends on the method performance and the results obtained from a new method must be better (e.g., more reliable, more consistent, a better turn-around time, improved sensitivity and/or specificity) or at minimum comparable, to the original method. Hemoglobin A1C (HbA1C) is used to illustrate an example of a method comparison (validation) in this article and eight steps demonstrate the process, described below.

1. Statement of Primary Laboratory Test Objectives

Method validation is performed when a lab acquires a new instrument and is testing a new procedure; it is used for assessing if a new instrument is reporting valid results. Using statistics to determine accuracy, a method validation can establish the instrument performance compared to a gold standard method. Analyzing quality control samples helps determine the instrument precision by calculating the mean, standard deviation (SD), and coefficient of variation (CV). Activities that can be involved in method validation studies include calibrations for various analytes and confirming the linearity of the method throughout the measurable range. The

performance of method validation studies are mandated by Clinical Laboratory Improvement Amendments (CLIA), the College of American Pathologists (CAP), and the Joint Commission for any new method.

2. Identify the Known Variables

The known variables are categorized as independent and dependent. They refer to characteristics of the patient sample. Independent variables include the substances in a sample such as lipemia, hemolysis, and icterus that can lead to erroneous results. Independent variables do not include the amount of the specimen. The dependent variable is the concentration of the analyte that will be used to determine a reference range.

3. Apply Appropriate Statistics

The statistical data such as the CV, SD, mean, random error (RE), systematic error (SE) are used to determine the method precision, accuracy, and total allowable error (TEa). The mean is calculated to obtain an average value for all the test results, the SD is a measure of the spread of the test results, and the CV is used to compare the mean value to the standard deviation and measure the dispersion of the test results. The TEa of the new test method includes both random error and systematic error. Random error is the error that occurs as a result of chance, and systematic error follows a predictable pattern. The difference in test results between a new method and an old method should be less than or equivalent to the total allowable error. Regression analysis is used to compare two variables to determine if a linear relationship exists. The two variables consist of an independent variable (X) and a dependent variable (Y). Linear regression statistics are used to determine the quality of the relation between the two variables.

Test results for the new test method are dependent on the test results of the old test method. All of the data must lie close to and on the straight line.

4. Clarify the Analyte of Interest and Method Selection

The analyte under investigation should be mentioned in this section of the study. The analyte used for this method validation is HbA1C. It is used to measure glycemic control over a period of 3 months. A description of the methodology used in the demonstration of this validation study is provided in Tables 1 and 2.

5. Sample Selection

The quality of the results of the validation study is dependent on the number of data points collected and the range of the data compared to the measurable range. An ideal number of data points/samples is 40 although some laboratories opt to use 20 data points/samples. The samples are chosen at random and should be representative of the normal and abnormal population. The samples should cover the analytical measurement range of the test method. The method validation described in this article used 40 unidentified patient samples at the high, normal, and low concentration range of the reference interval for hemoglobin A1C.

6. Describe the Methods

HbA1C samples were run at the Y hospital laboratory on the Siemens Dimension Vista 1500, which is the project site, and at the X reference laboratory on the Cobas Integra Roche Modular 800. Both of these analyzers have different reference ranges for HbA1C due to the difference in methodologies. The Vista 1500 uses an immunoturbidimetric assay whereas the Cobas Integra

800 operates on a Spectrophotometric immunoassay. The manufacturer established reference range for HbA1C on the Vista 1500 is 4.2 to 6.3 percent while it is 4.0 to 5.6 percent on the Cobas Integra 800. Patients with HbA1C values of 5.6 to 7.4 are considered high-risk patients. Patients who have diabetes have a hemoglobin A1C of greater than or equal to 6.5 percent. Each method for the HbA1C method validation study was analyzed for the clinical performance characteristics such as the sensitivity, specificity, accuracy, precision, reportable range, and performance standards.

7. **Performing Data Analysis**

The test result values (data) obtained from the Y hospital laboratory and the X reference laboratory are plotted on graphs and observed for any outliers. The mean, SD, and CV are calculated to determine the precision of the method. The data collected from both methods are plotted on a graph; the Y-axis represents data from the new method, and the X axis represents data from the old method. The best fit line is drawn, and the slope and y-intercept are calculated. The slope is the line of best fit, and the y-intercept is the value at which the line crosses the y-axis. The correlation coefficient is calculated to determine the relationship between the data. Statistical analysis software can be used to graph the data, calculate the slope, intercept, and determine the correlation coefficient.

The performance of the method is determined by calculating the total allowable error which consists of both the systematic error and the random error. The total allowable error is the amount of error which is clinically acceptable.

8. **Explaining the Results**

Table 1 and Table 2 show the original raw data and the statistical analyses results obtained

from a correlation study. Regression analysis is used to compare two variables to determine if a

linear relationship exists. The two variables consist of an independent variable (X) and a

dependent variable (Y; described above). Test results for the new test method are dependent on

the test results of the old test method. All of the data must lie close to and on a straight line.[8]

The Deming Regression may be used since it is a model that finds the line of best fit for a two-

dimensional dataset (X and Y variables). It differs from simple linear regression because it

accounts for observation errors on both the X- and the Y- axis. To validate a new method, it

must demonstrate a statistical relationship to the method currently in use. The methods can be

considered statistically identical if: the slope is 1.00 (within 95% confidence) or the intercept is

0.00 (within 95% confidence) (Figure 1).

Figure 1: Hemoglobin A1C analyzed by the X hospital lab and Y hospital lab method. The

scattered Plot indicates the dimming regression relationship between X and Y methods. This

approach assumes that both the X and Y methods are subject to measurement error which is

different from linear regression, which considers that the two methods has no random

measurement errors. The Bias Plot is a scatter plot with X on the x-axis, and Y-X on the y-axis.

The ideal bias plot would have all points falling exactly on the zero line.

Alternate (Quantitative) Method Comparison

X Method Integra 800   Y Method Vista 1500
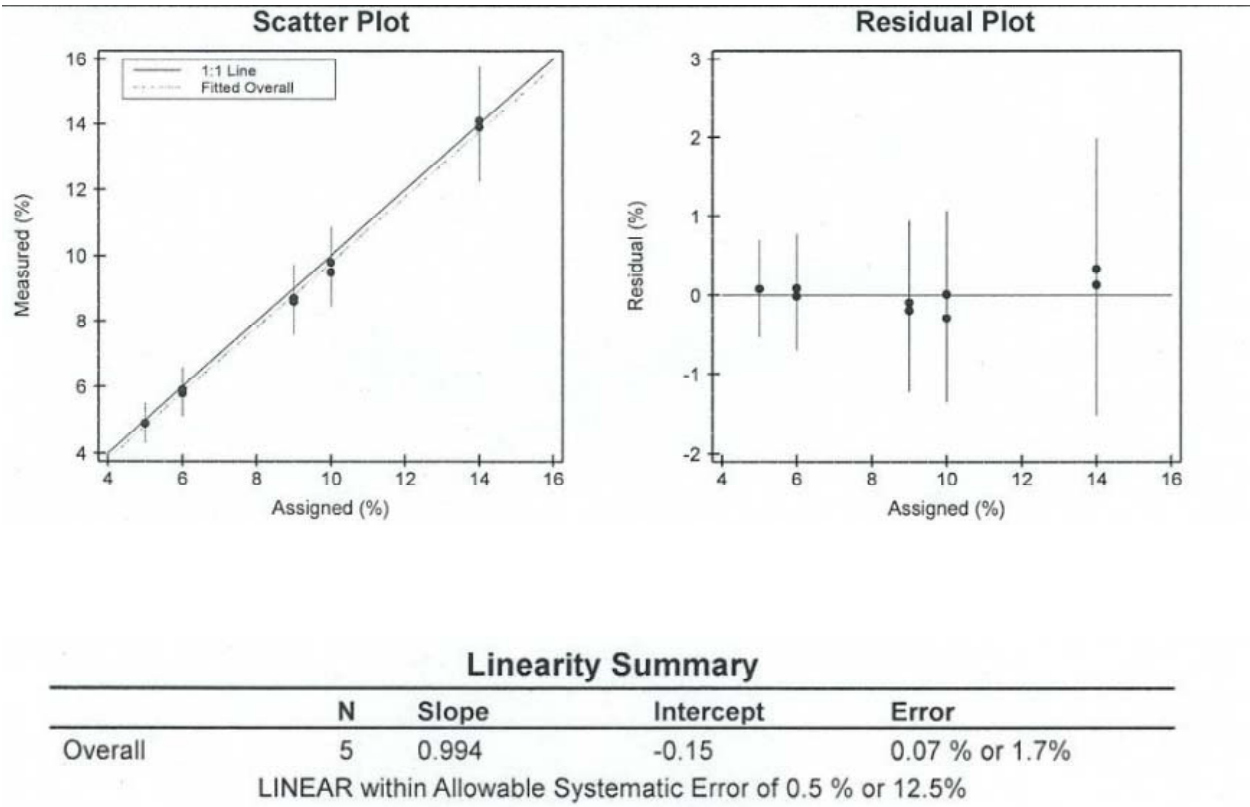

Scatter Plot
Bias
Percent Bias
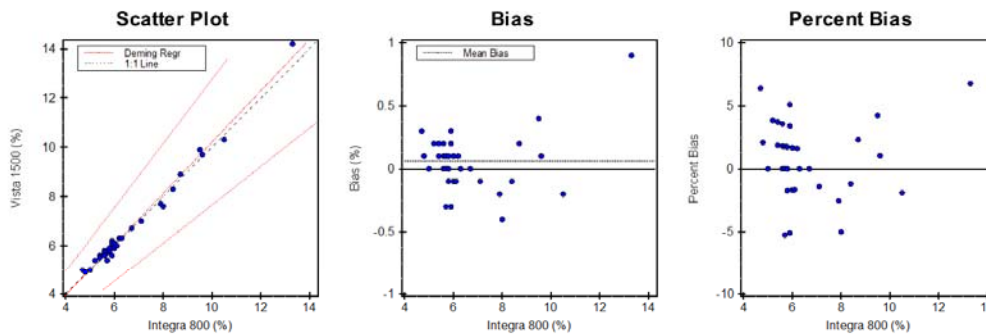
Regression Analysis

	Deming	Regular
Slope	1.042 (1.002 to 1.082)	1.035 (0.995 to 1.074)
Intercept	-0.21 (-0.48 to 0.06)	-0.16 (-0.43 to 0.10)
Std Err Est	0.21	0.21

95% Confidence Intervals are shown in parentheses

The results show a slope of m=1.042 and an intercept of -0.21, both values within 95% confidence interval indicating that the two methods are statistically identical. The TEa of 1 unit or 25% was used to determine if the method performance meets the acceptable standard error rate. The difference between the two methods was within the allowable error for 40 of 40 specimens (100%).

A serial dilution was performed to determine the linearity of the method throughout the reportable range. Six data points were collected for HbA1C by serial dilution starting at a concentration of 14 percent and diluting the sample to concentrations of 10, 9, 6, and 5 percent. The linearity module of the EP Evaluator program was used to verify the instrument reportable range (Figure 2). The method was proven linear throughout the reportable range.

Figure 2: The linearity of HbA1C which was analyzed on the chemistry Vista instrument. The Scatter Plot indicates the best fit line. The Residual Plot indicates the difference between the best fit line and either an individual result or a mean measured value.

Linearity Summary

	N	Slope	Intercept	Error
Overall	5	0.994	-0.15	0.07 % or 1.7%

LINEAR within Allowable Systematic Error of 0.5 % or 12.5%

The linearity of HbA1C was analyzed on the Vista over a measurement range of 4.90% to 14.00%. Allowable systematic error (SEa) was 0.5% or 12.5%. The systematic error is the amount of bias between the results of the new test method and the comparison method. All of the measured concentrations lie close to the mean and assigned values and are linear.

Summary

The eight steps used to validate a method help the laboratory ensure that a new test meets regulatory requirements. The example of the HbA1c study demonstrates an excellent correlation between the new and the old method. The study, however, did not include enough data points at

the lower and higher reportable range. More samples must be included to validate the

performance of the instruments at the lower and upper limits of the reportable range.

Table 1: HbA1C data for the test method (Y) and the comparison method (X) (N=40)

Alternate (Quantitative) Method Comparison

X Method Integra 800   Y Method Vista 1500

Experimental Results

		Results			Calc'd	SEE			Results			Calc'd	SEE
	Spec ID	X	Y	Bias	Y	Factor		Spec ID	X	Y	Bias	Y	Factor
1	S00012	4.7	5	0.3	4.69	1.5	21	S00027	5.9	6.2	0.3	5.94	1.2
2	S00015	4.8	4.9	0.1	4.79	0.5	22	S00031	5.9	5.6	-0.3	5.94	-1.6
3	S00003	5	5	0	5.00	0.0	23	S00032	5.9	6.1	0.2	5.94	0.8
4	S00010	5.2	5.4	0.2	5.21	0.9	24	S00028	6	5.9	-0.1	6.04	-0.7
5	S00035	5.4	5.5	0.1	5.42	0.4	25	S00009	6	6.1	0.1	6.04	0.3
6	S00020	5.4	5.6	0.2	5.42	0.9	26	S00018	6.1	6	-0.1	6.14	-0.7
7	S00022	5.6	5.8	0.2	5.62	0.8	27	S00019	6.2	6.3	0.1	6.25	0.2
8	S00036	5.6	5.6	0.0	5.62	-0.1	28	S00024	6.2	6.3	0.1	6.25	0.2
9	S00034	5.6	5.8	0.2	5.62	0.8	29	S00008	6.3	6.3	0.0	6.35	-0.3
10	S00026	5.6	5.7	0.1	5.62	0.4	30	S00013	6.3	6.3	0.0	6.35	-0.3
11	S00017	5.7	5.4	-0.3	5.73	-1.6	31	S00005	6.7	6.7	0.0	6.77	-0.3
12	S00025	5.7	5.8	0.1	5.73	0.3	32	S00023	7.1	7	-0.1	7.19	-0.9
13	S00011	5.7	5.7	0.0	5.73	-0.1	33	S00039	7.9	7.7	-0.2	8.02	-1.5
14	S00016	5.8	5.9	0.1	5.83	0.3	34	S00001	8	7.6	-0.4	8.12	-2.5
15	S00021	5.8	5.8	0.0	5.83	-0.2	35	S00040	8.4	8.3	-0.1	8.54	-1.1
16	S00006	5.8	5.9	0.1	5.83	0.3	36	S00029	8.7	8.9	0.2	8.85	0.2
17	S00033	5.8	5.9	0.1	5.83	0.3	37	S00030	9.5	9.9	0.4	9.69	1.0
18	S00007	5.8	5.9	0.1	5.83	0.3	38	S00038	9.6	9.7	0.1	9.79	-0.4
19	S00004	5.8	5.7	-0.1	5.83	-0.6	39	S00002	10.5	10.3	-0.2	10.73	-2.0
20	S00014	5.8	5.9	0.1	5.83	0.3	40	S00037	13.3	14.2	0.9	13.65	2.6

Values marked with an "X" were excluded from the calculations.

Table 2: Summary of the data statistics and a brief description of the hemoglobin A1C method comparison.

Supporting Statistics

Corr Coef (R)	0.9933	Points (Plotted/Total)	40/40
Bias	0.06 (0.96 %)	Outliers	Not Tested
X Mean ± SD	6.53 ± 1.73	Scatter Plot Bounds	Allowable Total Error
Y Mean ± SD	6.59 ± 1.80		1.00 % (conc) or 25.0%
Std Dev Diffs	0.22		
SubRange Bounds	None		

Experiment Description

	X Method	Y Method
Expt Date	21 Dec 2017	21 Dec 2017
Rep SD	1	1
Result Ranges	4.7 to 13.3	4.9 to 14.2
Units	%	%
Reagent	--	--
Calibrators	--	--
Analyst	WH	WH
Comment		

References

1. Alcala, H.G. et al., Effect of the method to measure levels of glycated hemoglobin on individual clinical decisions, Comparison of an Immunoassay with High-Performance Liquid Chromatography, Am. J. of Clin. Pathol., 2009; 132:332-335.

2. Bode, B.W., et al., Advances in Hemoglobin A1C Point of Care Technology, J. Diabetes Sci. Technol. 2007 May; 1(3): 405-411.

3, Bruneel, A., et al., External evaluation of the Dimension Vista 1500 intelligent lab system, J. Clin. Lab. Anal., 2012; 26(5): 384-97.

4. Chung, C., Xue, H., Pang,E., Tam, D. Accuracy of fasting plasma glucose and hemoglobin A1c testing for the early detection of diabetes: A pilot study, 2017; 1(2).

5. Clinical Chemistry and Immunochemistry Analyzers, Siemens Healthcare Diagnostics, 2011, retrieved from http://www.va.gov/nac/forms/Chemistry_CPRR_FSS_42008_Vista.pdf.

6. EP-Evaluator Online: Web-based Clinical Laboratory, Data Innovations LLC, 2013, retrieved from http://eeonline.datainnovations.com/cgi-bin/framework.exe?PG=StatMods

7. Fleming, J.K., Evaluation of HbA1c on the Roche Cobas Integra 800 closed-tube system, Clinical Biochemistry, 2007; 40(11): 822-7.

8. Gupta, S., Jain, U., Chauhan, N. Laboratory Diagnosis of HbA1c: A Review. J. Nanomed. Res., 2017; 5(4).

9. Hawkins, R.C., Laboratory Turnaround Time, Clin., Biochem. Rev., 2007; 28(4): 179-194.

10. Hemoglobin A1C Test, Home Health Testing, 2015, retrieved from: http://www.homehealthtesting.com/hemoglobin-a1c-test-p-38.html.

11. Huber, L., Validation of Analytical Methods, Agilent Technologies, 2010, retrieved from: https://www.agilent.com/cs/library/primers/Public/5990-5140EN.pdf

12. Joslin Diabetes Center, Stay healthy with Diabetes, All about A1C, 2015, retrieved from: http://www.joslin.org/info/all_about_a1c.html

13. Ladyzynski, P. et. al., Validation of a hemoglobin A1c model in patients with type 1 and type 2 diabetes and its use to go beyond the averaged relationship of hemoglobin A1c and mean glucose level, J. of Translational Medicine, 2014, 12:328.

14. Little, R.R., Roberts, W. L., A Review of Variant Hemoglobin's interfering with Hemoglobin A1c measurement, J. of Diabetes Science and Technology, 2009; 3(3):446-451.

15. Mertens, D., Roche HbA1c Testing Solutions: Setting a new standards in routine HbA1c testing, 2012, retrieved from: http://bvvietnamcubadonghoi.onlineoffice.vn/DOCUMENT/UPLOAD/1_TACNGHIEP/07_DU LIEUCONGTAC/hienxn/TAI_LIEU_KHOA/2._Roche_HbA1c_testing_solution_Dennis_Merte ns_EN.pdf

16. Molinaro, R., PhD, MT (ASCP), Professional Practice in Clinical Chemistry, Method Validation, Emory University, retrieved from: https://www.aacc.org/~/media/files/meetings-and-

events/resources-from-past-events/conferences/2013/professional-practice/april-28/mv_method_validation_apr_28_2013.pdf?la=en

17. Seaberg, R.S., Stallone, R.O., Statland, B.E., The role of total laboratory automation in a consolidated laboratory network, Clinical Chemistry, 2000; 46:5:751-756.

18. Shin, D.H., Development of an integrated reporting system for verifying hemolysis, icterus and lipemia in clinical chemistry results, Ann Lab Med. 2014 Jul; 34(4): 307–312.

19. Siemens, Laboratory Diagnostics Education and Training, retrieved from:

http://usa.healthcare.siemens.com/education/lab-education

20. Siemens, Dimension Vista 1500 Intelligent Lab System, retrieved from:

http://usa.healthcare.siemens.com/integrated-chemistry/systems/dimension-vista-1500-intel-lab-sys

21. Siemen's manufacturer inserts for the Dimension Vista system, Siemen's Healthcare Diagnostics Incorporation, 2015, retrieved from: www.siemens.com/diagnostics

22. Theodorsson, T., Validation and verification of measurement methods in clinical chemistry, Bioanalysis (2012) 4(3), 305–320.

23. Thevarajah, T.M. et. al., A comparison of three methods of measuring plasma glycosylated hemoglobin, Asian Biomedicine, Vol. 2, No.1, 2008; 43-49.

24. Westgard J., Basic Method Validation, 3rd edition, 2008.